

COMPARATIVE EFFICACY OF IR3535 AND DEET AS REPELLENTS AGAINST ADULT *Aedes aegypti* AND *Culex quinquefasciatus*

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ABSTRACT. Arm-in-cage laboratory evaluations of 2 proprietary formulations of the mosquito repellents IR3535 and *N,N*-diethyl-3-methylbenzamide (deet; aqueous cream, hydroalcoholic spray) were made with 10 and 20% concentrations of each repellent. Also, 4 commercially available products containing IR3535 (Expedition[®] insect repellent 20.07% active ingredient [AI], Bug Guard Plus with SPF30 sunscreen 7.5% AI, Bug Guard Plus with SPF15 sunscreen 7.5% AI, and Bug Guard Plus 7.5% AI) were tested. All comparisons were made on an equal formulation or concentration basis. Eight volunteers tested all formulations or products 3 times against laboratory-reared, *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (6–10 days old). Products were applied to a forearm at the rate of 0.002 g/cm². The other forearm was not treated and served as a control. Elapsed time to 1st and 2nd consecutive bite was recorded. Mean protection time (i.e., time to 1st bite) with proprietary formulations of IR3535 were comparable to those of deet, with 20% concentrations providing greater protection against *Ae. aegypti* (3 h) and *Cx. quinquefasciatus* (6 h). Mean protection time for commercial products containing IR3535 ranged from nearly 90 to 170 min for *Ae. aegypti* and 3.5 to 6.5 h for *Cx. quinquefasciatus*. Mean time to the 2nd bite was similar to time to 1st bite for each mosquito species, product, and formulation.

KEY WORDS Deet, IR3535, repellent, mean time to bite, personal protection

INTRODUCTION

Health officials recommend that personal protection from mosquito bites warrants the wearing of protective clothing, avoiding areas where mosquitoes develop (including periods of the day when mosquitoes are most active), and using insect repellent (Barnard 2000, CDC 2003a). Moreover, repellents are viewed as “critical public health tools” to minimize the transmission of arthropod-borne diseases (Osimitz and Grothaus 1995). However, a variety of synthetic and natural substances continues to enter the marketplace, often with unsubstantiated claims of superior repellency against biting arthropods. Several studies and reviews evaluating these products against adult mosquitoes have indicated that *N,N*-diethyl-3-methylbenzamide (deet) remains the most effective mosquito repellent and is often considered the gold standard to which other repellent products should be compared (Schreck 1996, Chou et al. 1997, Fradin 1998, Fradin and Day 2002). However, there is considerable public interest in identifying alternatives to this compound.

In 1999, the U.S. Environmental Protection Agency (EPA) approved for use in the United States the synthetic insect repellent known as IR3535 (ethyl butylacetylaminopropionate), which is structurally based on a natural amino acid, β -alanine (EPA 1999). This compound has been used as an insect repellent against a variety of blood-feeding arthropods without substantial adverse effects in Europe for >20 years and in Asia for nearly 10 years (Marchio 1996). Since 1957, deet has been registered for use by the general public. Although its use has been extensive, with a remarkable safety

profile, periodic toxic and allergic reactions have been reported, often from misapplication (Reuveni and Yagupsky 1982, Osmitz and Grothaus 1995, Qiu et al. 1998, Barnard 2000, Fradin 1998). No toxicity issues have been associated with IR3535, and it has been shown not to be harmful when ingested, inhaled, or used on skin (EPA 1999). Despite its long-standing history of safety in other parts of the world (Combemale et al. 1992), IR3535 has been met with limited acceptance in the United States. In an attempt to identify and provide guidance to the general public on alternative materials for protection against mosquito bites, we compared the mean protection times of several proprietary and commercial formulations of deet and IR3535 using 2 species of adult mosquitoes. *Aedes aegypti* (L.) was selected for its global role as the vector responsible for yellow fever and dengue. *Culex quinquefasciatus* Say was used because it is a vector of St. Louis encephalitis and, most recently, a vector of West Nile virus in North America (Sardelis et al. 2001).

METHODS

Products: Identical aqueous cream and hydroalcoholic spray formulations containing either IR3535 or deet at concentrations of 10 and 20% active ingredient (AI) were formulated by, and received from, EMD Chemicals, Inc. (Hawthorne, NY)/Merck KGaA (Darmstadt, Germany) for testing. In a 2nd study, the following commercially available Avon Skin-So-Soft products were evaluated: Expedition[®] insect repellent (20.07% IR3535, pressurized aerosol spray), Bug Guard Plus with SPF30 sunscreen (7.5% IR3535, lotion), Bug Guard

Plus with SPF15 sunscreen (7.5% IR3535, spray), and Bug Guard Plus (7.5% IR3535, spray). Unless specifically stated, spray formulations were delivered via a nonpressurized aerosol pump spray. All products were identified by codes, to which only the senior author had access.

Testing methods: Eight volunteers (5 men and 3 women) from the staff of the John A. Mulrennan Sr. Public Health Entomology Research and Education Center at Florida A&M University (Panama City, FL) participated in all tests. Before evaluations commenced, the total surface area of each volunteer's forearm (bend of elbow to wrist) was calculated by the following equation to determine the frustrum of a right circular cone (Miller et al. 2001).

$$(\text{radius of wrist} + \text{radius at bend of elbow}) \times \text{length of forearm} \times \pi$$

The total surface area was then multiplied by the rate of application, 0.002g/cm², to determine the total amount of repellent to be applied. This was done to standardize repellent application and prevent bias from variation in forearm size.

On the day of testing, all volunteers washed both forearms with an unscented soap (Ivory®), dried with a paper towel, then swabbed down with 70% ethanol. Each forearm was allowed to air dry for about 5 min before application. Creams were applied topically with a metal spatula (surface 0.8 × 5.5 cm), whereas pump sprays were applied approximately 6 cm from the surface of the forearm. Care was taken to ensure uniform coverage of each formulation or product at the rate of 1 g per 500 cm² of skin surface area. The other forearm of each volunteer was not treated and served as a nontreated control. An initial 10-min waiting period was used to "age" each repellent before evaluations began. This allowed formulations to be absorbed by the skin's surface. We also believe it minimized cage contamination by volatiles and direct contact of cage surfaces with a freshly treated (i.e., "wet") arm. Both hands were left untreated and were covered with powder-free latex gloves (secured by rubber bands) to prevent mosquitoes from biting them while in the test cages. New gloves were used at the start of each test interval. All volunteers were directed not to touch or rub the treated arm between evaluation periods.

Disease-free, non-blood fed, laboratory-reared adult *Ae. aegypti* and *Cx. quinquefasciatus* female mosquitoes (7–10 days old) were used in all evaluations. Both species were reared in rooms maintained at 24–33°C at 60–75% relative humidity and with a 12:12 h light:dark cycle. Prior to testing, adults were maintained on 10% sucrose-saturated cotton pads for at least 24 h. Because *Cx. quinquefasciatus* are fairly timid biters, sugar solution was withheld from their cages for about 16 h before testing commenced to prevent interference with host seeking. Sugar solutions were not removed

from *Ae. aegypti* because the presence or absence of carbohydrates did not appear to diminish biting activity in earlier evaluations. During tests, mosquitoes had access to a cotton pad (5 × 5 cm) saturated with only water in each cage.

Fifty mosquitoes of each species were released into individual metal cages (33 × 25.5 × 25.5 cm). Each cage had a solid clear plexiglass top for viewing purposes. One end of each cage was screened, whereas the other end was fit with a tubular stockinette (Owens and Minor, Jacksonville, FL) to allow introduction of mosquitoes and insertion of a forearm for evaluation. Care was taken to minimize contact of a treated forearm with the stockinette. In addition, a single layer of paper was placed in the bottom of each cage to minimize further contamination.

In this study, time to bite was the criterion used to determine the effectiveness of a repellent. The testing protocol generally followed that of Fradin and Day (2002), in which each formulation or product was evaluated at 2, 10-min intervals for the 1st 20 min then continued at intervals of 15 min for 4 h if no bite was recorded. Time to 1st bite was recorded when at least 1 bite was observed during a time interval. Testing ended if a 2nd bite occurred at the next consecutive time interval. If a bite was not observed after 4 h, testing continued at 30-min intervals for an additional 4 h. If a bite was recorded during the 30-min intervals, the evaluation period was shortened to 15 min until a 2nd consecutive time interval with a bite was recorded. Consecutive time intervals with a bite were used to reduce variability from random biting events not related to direct protection from the repellent. Testing concluded for that day if no bites were recorded on the treated forearm at 8 h. Testing ended when 2 consecutive time intervals resulted in at least 1 bite during each interval. Temperature and humidity were recorded during all test intervals.

At hourly intervals, a new group of 50 mosquitoes, not exposed to a repellent, was used for the control arm. The previous cage used for controls was then used for testing the treated arm. An hourly rotation was chosen because it was the minimum amount of time needed to replace the stockinette and paper on the cage bottom (to prevent cross-contamination between treatments and controls) before introduction of a new set of mosquitoes for the next test interval.

During each test, the receptiveness of mosquitoes to obtain a blood meal was evaluated by mosquito landings on the control arms at each time interval (Fradin and Day 2002). In addition, time to 1st bite on control arms was periodically recorded as an additional measurement of mosquito attractiveness. No more than 1 mosquito was allowed to bite control arms when recording time to 1st bite. Because *Ae. aegypti* is a very aggressive biter, control arms were placed in cages for 30 sec, whereas repellent-treated arms were placed in cages for 1 min. Con-

Table 1. Mean \pm SE¹ time to 1st bite for 2 species of adult mosquitoes after exposure to forearms treated with 4 formulations of IR3535 and deet in laboratory tests ($n = 24$).

Product	Active ingredient (%)	Cream		Spray	
		Time (min)	Range (min)	Time (min)	Range (min)
<i>Aedes aegypti</i>					
IR3535	20	179.7 ± 15.4 a	105–285	167.3 ± 12.3 a	80–320
IR3535	10	116.7 ± 10.3 b	50–230	121.3 ± 11.7 b	64–320
deet	20	197.0 ± 15.3 a	95–350	176.2 ± 13.6 a	105–380
deet	10	131.3 ± 11.0 b	50–240	156.9 ± 14.2 a	65–290
<i>Culex quinquefasciatus</i>					
IR3535	20	393.8 ± 22.0 a	185–480	390.0 ± 24.2 a	170–480
IR3535	10	344.4 ± 26.6 a	110–480	325.0 ± 25.0 b	125–480
deet	20	370.2 ± 22.5 a	185–480	366.0 ± 23.4 ab	195–480
deet	10	341.9 ± 26.8 a	115–480	342.9 ± 24.6 ab	170–480

¹ Means in each column for each species, product, and formulation (cream or spray) followed by the same letter are not significantly different ($P > 0.05$) by the Student-Newman-Keuls test.

versely, *Cx. quinquefasciatus* are fairly timid biters, so evaluation periods for control and treatments were 2 min each. Each proprietary and commercial formulation was evaluated on 3 different days for each volunteer, yielding a total of 24 tests conducted for each formulation. Repellent formulations were assigned randomly to each participant, and no one tested >1 formulation per day.

Statistical analysis: The study incorporated a randomized complete block design in assigning formulations to each volunteer for testing on each day. Prior to analyses, data for each mosquito species were transformed by $\log(x + 1)$ in an attempt to reduce heteroscedasticity in time to bite (Steel and Torrie 1980). Transformed data were then subjected to a 2-way ANOVA (subject and repellent formulation) followed by a Student-Newman-Keuls test to separately compare the mean time to 1st bite ($P < 0.05$) for cream and sprays of each active ingredient (Cochran and Cox 1957, SAS Institute 1990). Proprietary formulations and commercial formulations were compared separately. Time to 2nd bite

data followed the same analyses. Tables show non-transformed means.

RESULTS

The host-seeking "aggressiveness" of each mosquito species differed greatly on control arms. Time to 1st bite for *Ae. aegypti* averaged 9.8 ± 0.3 sec, whereas *Cx. quinquefasciatus* averaged 41.0 ± 1.1 sec.

Mean protection time against the 1st bite for IR3535 and deet 20% cream formulations were similar for *Ae. aegypti* and averaged about 180 min (Table 1). However, time to 1st bite was significantly lower for each repellent when 10% AI was compared with 20% AI. There was no significant difference ($P > 0.05$) in time to 1st bite between repellent type and concentration when cream formulations were tested against *Cx. quinquefasciatus*. Mean protection time was considerably greater for this species than *Ae. aegypti* and averaged about 6 h.

Table 2. Mean \pm SE¹ time to 2nd consecutive bite for 2 species of adult mosquitoes after exposure to forearms treated with 4 formulations of IR3535 and deet in laboratory tests ($n = 24$).

Product	Active ingredient (%)	Cream		Spray	
		Time (min)	Range (min)	Time (min)	Range (min)
<i>Aedes aegypti</i>					
IR3535	20	183.9 ± 14.3 a	105–432	172.7 ± 12.4 a	80–320
IR3535	10	122.9 ± 10.1 b	65–245	126.9 ± 12.2 b	64–320
deet	20	202.0 ± 15.0 a	105–350	183.1 ± 14.2 a	105–395
deet	10	138.8 ± 12.1 b	50–255	160.6 ± 14.7 a	65–290
<i>Culex quinquefasciatus</i>					
IR3535	20	397.3 ± 21.2 a	185–480	397.5 ± 22.1 a	170–480
IR3535	10	348.2 ± 26.1 a	125–480	328.8 ± 24.6 b	125–480
deet	20	375.6 ± 21.8 a	185–480	371.7 ± 22.4 ab	215–480
deet	10	346.9 ± 26.3 a	115–480	346.7 ± 24.1 ab	185–480

¹ Means in each column for each species, product, and formulation (cream or spray) followed by the same letter are not significantly different ($P > 0.05$) by the Student-Newman-Keuls test.

Table 3. Mean \pm SE¹ time to 1st bite for 2 species of adult mosquitoes after exposure to forearm treated with 4 over-the-counter Avon Skin-So-Soft formulations containing IR3535 in laboratory tests ($n = 24$).

Product	Active ingredient (%)	Formulation	<i>Aedes aegypti</i>		<i>Culex quinquefasciatus</i>	
			Time (min)	Range (min)	Time (min)	Range (min)
Expedition	20.07	Aerosol spray	125.0 \pm 11.8 a	90–305	351.7 \pm 22.6 a	240–480
Bug Guard plus SPF30 sunscreen	7.5	Cream	169.4 \pm 12.0 b	50–240	356.5 \pm 19.8 a	110–480
Bug Guard plus SPF15 sunscreen	7.5	Pump spray	111.9 \pm 10.2 a	35–155	255.2 \pm 20.8 b	140–480
Bug Guard	7.5	Pump spray	84.0 \pm 7.3 c	65–320	226.7 \pm 18.0 b	210–480

¹ Means in each column for each species followed by the same letter are not significantly different ($P > 0.05$) by the Student-Newman-Keuls test.

Time to 1st bite for IR3535 and deet sprays, at both concentrations, were similar for *Ae. aegypti* and averaged 157 min, with the exception of 10% IR3535, which was significantly less at 120 min (Table 1). *Culex quinquefasciatus* showed similar efficacy for deet and IR3535, with mean times to 1st bite ranging from 325 to 390 min. Mean protection time to 2nd bite was similar as time to 1st bite for both mosquito species, products, and formulations (Table 2).

For the commercial products, mean protection time against bites of *Ae. aegypti* was greatest for Bug Guard with SPF30 sunscreen (170 min) compared with the other Avon products (Table 3). Time to 1st bite for Expedition and Bug Guard with SPF15 sunscreen were not significantly different from each other and averaged about 2 h. However, Bug Guard spray without sunscreen gave the least protection (84 min) against bites when compared with the other 3 products.

Time to 1st bite for *Cx. quinquefasciatus* was significantly greater for Expedition and Bug Guard with SPF30 sunscreen (about 6 h) compared with the other 2 products (Table 1). Times to 1st bite for Bug Guard with SPF15 sunscreen and Bug Guard spray without sunscreen were not significantly different from each other and averaged about 4 h. Time to 2nd bite followed the exact pattern as time to 1st bite for both mosquito species and all products (Table 4).

DISCUSSION

Mean protection times afforded by the cream and spray proprietary formulations of IR3535 and deet against bites from *Ae. aegypti* and *Cx. quinquefasciatus* were generally similar when compared on an equal formulation or concentration basis. Commercial products exhibited a wide variance in protection times but remained similar to proprietary formulations. Other workers have found that 20% concentrations of deet and IR3535 each provided about 9 and 13 h of protection from *Ae. aegypti* and *Culex tritaeniorhynchus* Giles bites, respectively, in laboratory trials (Thavara et al. 2001).

We also observed that protection time from bites of *Cx. quinquefasciatus* was about double that of *Ae. aegypti*. This observation is similar to other reports for deet protection times and might be explained by the avidity in which each mosquito species obtains a blood meal (Yap et al. 2000). That is, *Ae. aegypti* appears to be a very aggressive host-seeking mosquito species because it is primarily anthropophilic, whereas *Cx. quinquefasciatus*, although primarily ornithophilic, will occasionally feed on humans (Mullen and Durden 2002).

However, mean protection times should only be used as a reflection of relative effectiveness. Previous studies by other workers have shown that mean protection times can differ considerably within and between studies. One study reported about

Table 4. Mean \pm SE¹ time to 2nd consecutive bite for 2 species of adult mosquitoes after exposure to forearms treated with 4 over-the-counter Avon Skin-So-Soft formulations containing IR3535 in laboratory tests ($n = 24$).

Product	Active ingredient (%)	<i>Aedes aegypti</i>			<i>Culex quinquefasciatus</i>		
		Formulation	Time (min)	Range (min)	Time (min)	Range (min)	
Expedition	20.07	Aerosol spray	132.9 \pm 12.0 a	90–320	353.5 \pm 22.4 a	225–480	
Bug Guard plus SPF30 sunscreen	7.5	Cream	175.0 \pm 11.9 b	35–200	363.1 \pm 20.5 a	150–480	
Bug Guard plus SPF15 sunscreen	7.5	Pump spray	118.3 \pm 10.5 a	50–170	260.2 \pm 20.5 b	140–480	
Bug Guard	7.5	Pump spray	87.1 \pm 7.2 c	65–335	230.4 \pm 17.7 b	210–480	

¹ Means in each column for each species, product, and formulation (i.e., cream or spray) followed by the same letter are not significantly different ($P > 0.05$) by the Student-Newman-Keuls test.

6 h protection time against bites of *Ae. aegypti* with a 10% deet-alcohol application, but in our study, we found it to be only about half that time (Yap et al. 2000). Another study reported protection times of 9 h for *Ae. aegypti* with a 20% alcoholic preparation of IR3535 (Thavara et al. 2001), whereas we showed an average of 167 min of protection against this species. Conversely, a recent study found that Avon Skin-so-Soft's Bug Guard Plus (7.5% IR3535) provided only 24 min of mean protection from *Ae. aegypti* bites, whereas we observed 84 min of protection for this formulation (Fradin and Day 2002). We also found that those commercial products that contained sunscreen significantly increased repellency compared with products without it. It is unknown whether the sunscreen is repellent, provides a skin barrier to host location, enhances the repellency of the active ingredient by additive or synergistic properties, or lowers evaporation rate of the repellent from the skin surface.

The relative difference of protection times, in our and earlier repellent efficacy studies, could be linked to variation in several factors, including environmental conditions, mosquito species, test subjects, formulation chemistries, application techniques, and study design (Schreck 1977, Barnard 1998, Golenda et al. 1999, Fradin and Day 2002). Indeed, we found that the test subject was a significant ($P < 0.01$) source of variation in our studies regardless of formulation or mosquito species tested.

Although laboratory testing provides a general indicator for product efficacy, several factors, such as reduced product evaporation and lotion breakdown rates strongly limit their application outside this realm. However, field tests remain the benchmark for establishing true efficacy of an insect repellent and remains the only testing requirement for EPA approval of such products (EPA 1999, 2000).

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